Determining the efficacy of nanoparticle tracking to observe biofilm breakdown

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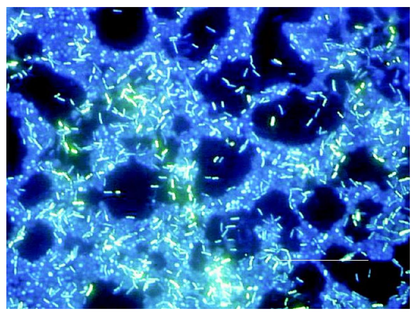
Motivation and Aims

Motivation

Biofilm formation and breakdown is of concern in many hydrated systems.

- Water distribution pipes
- Surgical and medical appliances
- Ship hulls
- Dental plaque
- Urinary catheters
- Bathroom sinks and showers



Stained biofilm grown for 14 days. Donlan epifluorescence microscopy (20 μm) image. Bacteria in yellow, polysaccharide matrix in blue, and voids and channels in black.

The National Institutes of Health state that 65% of microbial and 80% of chronic infections are associated with biofilm formation.

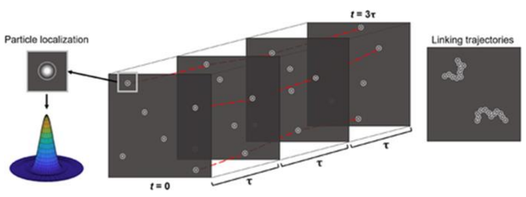
Research Aims

1. Develop a method to image nanoparticles in biofilms.
2. Determine if additives induce a breakdown in biofilm structure by measuring nanoparticle diffusion.

Donlan. *Emerg. Infect. Dis*. **2002**.

Methods and Materials

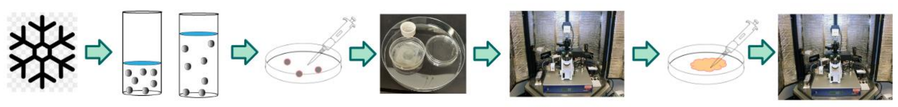
Single Nanoparticle Tracking



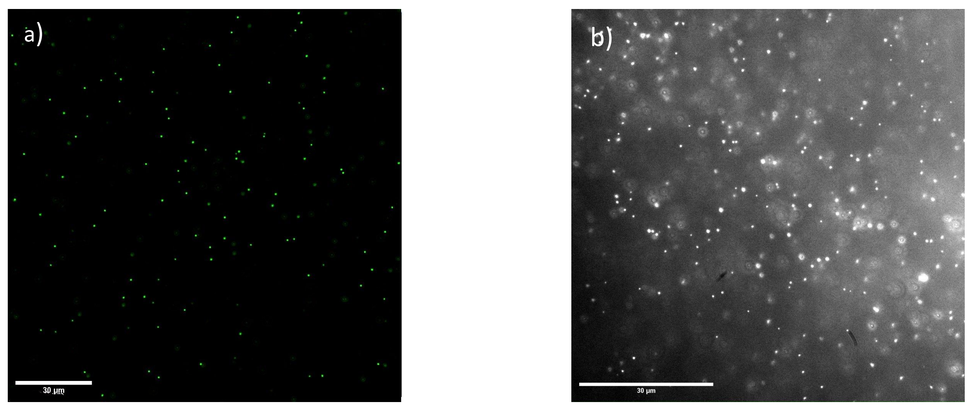
Particle tracking of fluorescent nanoparticles can help determine the structural breakdown of a biofilm over time.

Experimental Setup for Tracking with Addition of Positive Control

1. Remove biofilm from freezer.
2. Dilute nanoparticles in buffer.
3. Pipette nanoparticles onto biofilm.
4. Allow nanoparticles to infiltrate oral biofilm for two days.
5. Image on TIRF.
6. Add positive control.
7. Image on TIRF at 2 min intervals for 1 hr.



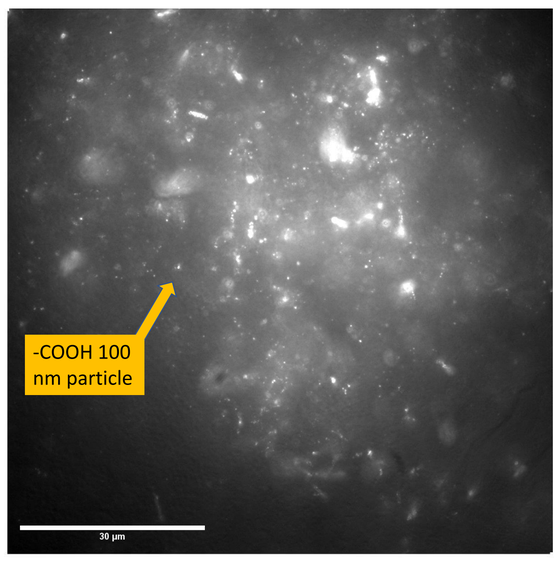
Imaging Techniques



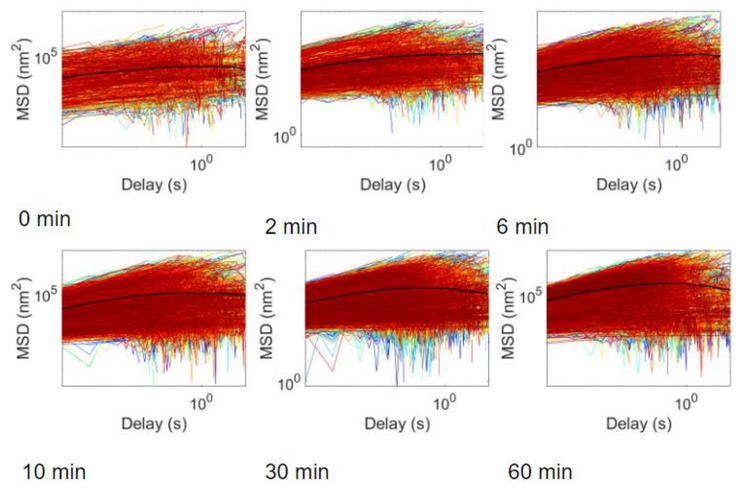
Video frames of -COOH green fluorescent particles in 80% glycerol water were compared using a (a) confocal microscope with 500 nm particles alongside (b) a total internal reflection (TIRF) with 100 nm particles and it was found that the TIRF was able to capture the higher frame rate.

Results

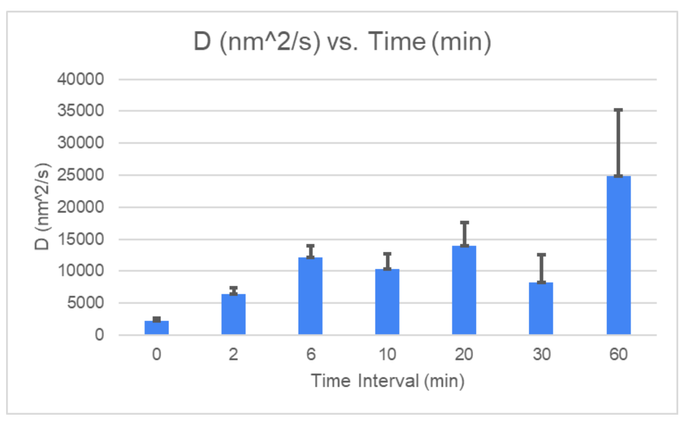
a) Biofilm with Nanoparticles



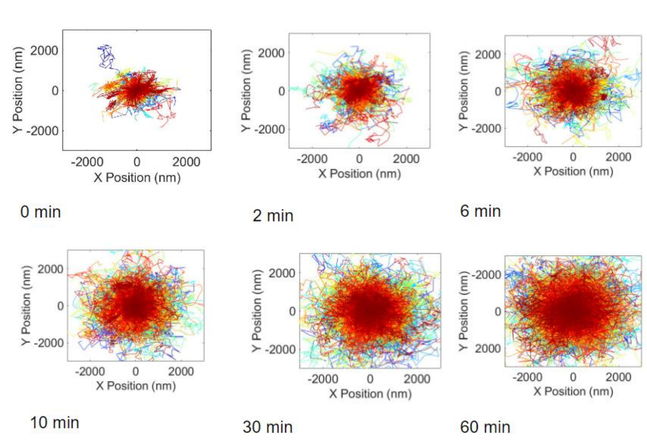
b) MSD plots of TIRF videos



d) Determination of Diffusivity



c) XY Positions of TIRF videos



Single nanoparticle tracking can be analyzed to determine the magnitude of biofilm breakdown as evidenced by a) single frame of nanoparticle infiltrated biofilm, b) mean squared displacement (MSD) plots of TIRF videos, c) XY plots of TIRF videos, and d) diffusivity values derived from linear weighted fit of the mean MSD curves over time of 100 nm -COOH functionalized nanoparticles in biofilm after the addition of positive control.

Conclusion

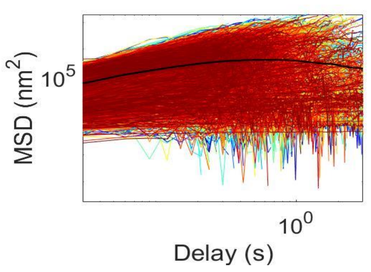
1. Diffusivity and the confidence interval generally increase over time, indicating the breakdown of the structure.

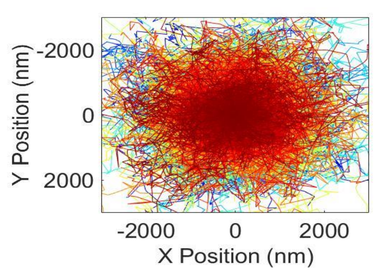
2. The heterogeneity of the biofilm structure perhaps increases as the biofilm breaks down as evidenced by the larger confidence interval.

3. Single nanoparticle tracking can be analyzed to determine if a biofilm is breaking down after addition of positive control additive.

Table I. Diffusivity of 100 nm -COOH functionalized green fluorescent nanoparticles in biofilm

Time Interval (min)	D (nm^2/s)	Confidence Interval
0	2.16E+03	5.00E+02
2	6.45E+03	1.00E+03
6	1.22E+04	1.83E+03
10	1.03E+04	2.49E+03
20	1.40E+04	3.66E+03
30	8.28E+03	4.21E+03
60	2.48E+04	1.04E+04





My Journey

1. Oak Ridge National Laboratory Summer Intern
2. Undergraduate Researcher and Teaching Assistant at University of Pennsylvania
3. Co-founder and COO of Baleena

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